

Electronic Supplementary Information (ESI)

Carbon Nitride-TiO₂ Hybrid Modified with Hydrogenase for Visible Light Driven Hydrogen Production

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Experimental Section

Materials. Chemicals were purchased from commercial suppliers and used without further purification. Reagents for the analytical part of the work were of the highest available purity. The [NiFeSe]-hydrogenase from *Desulfomicrobium baculatum* (*Dmb* [NiFeSe]-H₂ase) was purified by a previously published method.¹ The preparation has a specific activity of 2115 $\mu\text{mol H}_2 \text{ min}^{-1} \text{ mg}^{-1}$,² and the stock enzyme solution was diluted with an aqueous TEOA solution (0.1 M, pH 7) before photocatalytic experiments in an anaerobic glovebox. Polyheptazine (melon, CN_x) was prepared by heating melamine at 500 °C for 2 hours.³ Note that the CN_x employed in this study displayed the same activity than a previously used CN_x from a different source.⁴ CN_x-TiO₂ powders were synthesised as previously described.^{3b} In short, 0.5 g of TiO₂ (Hombikat UV 100, Sachtleben Chemie, Germany, anatase, specific surface area (BET) 300 m² g⁻¹, crystallite size < 10 nm) was placed into a Schlenk tube connected via an adapter to a round-bottomed flask containing urea (1 g), and heated in a muffle oven for 30 min at 425 °C. Powders were washed and centrifuged with water three times and then dried at 60 °C overnight, giving samples denoted as CN_x-TiO₂. The specific surface area (BET) of CN_x-TiO₂ and of CN_x was 111 m² g⁻¹ and 8 m² g⁻¹, respectively. CN_x-ZrO₂ was prepared using an analogous procedure with a urea heat treatment at 600 °C starting with ZrO₂ powders. ZrO₂ was prepared by basic hydrolysis (pH 9) of ZrOCl₂ (1 M) solutions, followed by filtration, drying, and calcination at 500 °C for 2 hours.

Photocatalysis Experiments. A standard photocatalytic experimental set-up was used as follows: CN_x-TiO₂ (5 mg) was added to a borosilicate glass tube containing a magnetic stir bar (total volume 7.74 mL). An aqueous electron donor solution (usually 0.1 M, 2.98 mL) was then added and the vessel sealed with a rubber septum. The suspension was sonicated for 20 min under air, followed by purging the suspension for 15 min with 2% CH₄ in N₂. *Dmb* [NiFeSe]-H₂ase was then added

(16.5 μL ; 3 mM) and then the vial was purged with 2% CH_4 in N_2 for an additional 5 min. The vials were then placed in a water-jacketed glass rack and irradiated with 1 sun using a Xe lamp (Newport Oriel Solar Light Simulator, 1000 W, 100 mW cm^{-2}) with an air mass 1.5 global (AM 1.5G) filter in the absence or presence of a 420 nm or 455 nm UV-broad band cut-off filters (UQG Optics), with stirring. Headspace gases were sampled using Hamilton air-tight syringes by injecting 20 μL into the gas chromatograph (Agilent 7890A Series gas chromatograph equipped with a 5 Å molecular sieve column, N_2 carrier gas 3 mL (min)^{-1} and an oven temperature of 45 °C and a thermal conductivity detector) at regular intervals. H_2 produced was quantified by comparison to the CH_4 internal standard.

Centrifugation Experiments. Photocatalytic experiments were set up by pre-loading the H_2ase (50 pmol) onto $\text{CN}_x\text{-TiO}_2$ (5 mg) by stirring the suspension under N_2 for 15 min in an aqueous EDTA solution (0.1 M, 1 mL). The suspension was then transferred to centrifuge tubes and spun down for 10 min (10,000 rpm). The supernatant was decanted (in air) and the pellet re-suspended in an aqueous EDTA solution (0.1 M, pH 6) and vortexed for 2 min. The suspension was returned to the borosilicate glass vials, sealed and purged with 2% CH_4 in N_2 for 10 min. The vessels were irradiated under 1 sun for 4 h and H_2 production monitored by gas chromatography.

Calculation of Hydrogenase Loading. The enzyme loading onto $\text{CN}_x\text{-TiO}_2$ was calculated based on the BET surface area of $111 \text{ m}^2 \text{ g}^{-1}$, a crystallite size of $r = 5 \times 10^{-9} \text{ m}$ and a conservative estimate that approximately one-quarter of the surface area of $\text{CN}_x\text{-TiO}_2$ is exposed TiO_2 that is accessible for the enzyme to adsorb. SA is surface area, A is Avogadro's number, n is the number of moles of hydrogenase used, m the number of particles of $\text{CN}_x\text{-TiO}_2$ used.

$$\begin{aligned}
\text{Number of CN}_x\text{TiO}_2 \text{ particles, } m &= \frac{(\text{BET of CN}_x\text{TiO}_2)(\text{g CN}_x\text{TiO}_2)(\% \text{ SA exposed TiO}_2)}{4\pi r^2} \\
&= \frac{(111 \text{ m}^2\text{g}^{-1})(0.005 \text{ g})(0.25)}{4\pi(5 \times 10^{-9})^2 \text{ m}^2} \\
&= 4.4 \times 10^{14} \text{ particles} \\
\therefore \text{Hydrogenase Loading} &= \frac{nA}{m \text{ CN}_x\text{TiO}_2 \text{ particles}} \\
&= \frac{(50 \times 10^{-12} \text{ mol H}_2\text{ase})(6.02 \times 10^{23} \text{ H}_2\text{ase mol}^{-1})}{4.4 \times 10^{14} \text{ particles}} \\
&= 0.1 \text{ H}_2\text{ase enzymes per particle of CN}_x\text{TiO}_2
\end{aligned}$$

Treatment of Data. All analytical measurements were performed at least in triplicate. A minimum standard deviation (σ) of 10% was assumed for all experiments even where triplicate runs gave a σ of less than 10%. The solar light source and the gas chromatograph were calibrated regularly to ensure reproducibility.

Calculation of External Quantum Efficiency (EQE). Hydrogen generation in a quartz cuvette containing excess H₂ase (50 pmol) with CN_x-TiO₂ (1.7 mg) suspended in EDTA (1 mL, 0.1 M, pH 6) was driven by blue light ($\lambda = 400 \pm 10$ nm) of intensity $I = 4.34 \text{ mW cm}^{-2}$ and UV light ($\lambda = 360 \pm$ nm) of intensity $I = 2.49 \text{ mW cm}^{-2}$ at 25 °C using narrow bandpass filters (THOR Labs). The EQE can be calculated with the following formula:

$$\text{EQE (\%)} = (2 \cdot n_{\text{H}_2} \cdot N_A \cdot h \cdot c) / (t_{\text{irr}} \cdot \lambda \cdot I \cdot A) \cdot 100$$

Where n_{H_2} is the moles of H₂ photo-generated, N_A is the Avogadro constant, h is the Planck constant, c is the speed of light, t_{irr} is the irradiation time, and A is the irradiated area of the photoreactor (1 cm²).

References

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- 2 E. C. Hatchikian, M. Bruschi and J. Le Gall, *Biochem. Biophys. Res. Commun.*, 1978, **82**, 451-461.
- 3 (a) B. V. Lotsch, M. Döblinger, J. Sehnert, L. Seyfarth, J. Senker, O. Oeckler and W. Schnick, *Chem. Eur. J.*, 2007, **13**, 4969-4980; (b) M. Bledowski, L. Wang, A. Ramakrishnan, O. V. Khavryuchenko, V. D. Khavryuchenko, P. C. Ricci, J. Strunk, T. Cremer, C. Kolbeck and R. Beranek, *Phys. Chem. Chem. Phys.*, 2011, **13**, 21511-21519.
- 4 C. A. Caputo, M. A. Gross, V. W. Lau, C. Cavazza, B. V. Lotsch and E. Reisner, *Angew. Chem. Int. Ed.*, 2014, **53**, 11538-11542.

Table S1. Solar light driven H₂ production with *Dmb* [NiFeSe]-H₂ase (50 pmol) on CN_x-TiO₂ (5 mg). Experiments were performed using CN_x-TiO₂ or other light harvesting particles (5 mg) in aqueous ascorbic acid (AA; 0.1 M), triethanolamine (TEOA; 0.1 M), EDTA (0.1 M) or citric acid (CA; 0.1 M) solution with H₂ase as a catalyst. Entry 6 represents optimised standard conditions under AM 1.5 G solar light irradiation ($\lambda > 300$ nm).

Entry	H ₂ ase / pmol; light absorber	pH	Electron donor	TON (4 h) $\pm \sigma$ / mol H ₂ H ₂ ase ⁻¹	H ₂ (4 h) $\pm \sigma$ / μ mol	Activity (1 h) / μ mol H ₂ (g CN _x -TiO ₂) ⁻¹ h ⁻¹	TOF (1 h) $\pm \sigma$ / h ⁻¹
Different Electron Donors (UV-vis irradiation)							
1	50; CN _x -TiO ₂	6	AA	762 \pm 72	0.04 \pm 0.01	5 \pm 1	516 \pm 52
2	50; CN _x -TiO ₂	10.5	TEOA	2378 \pm 234	0.12 \pm 0.01	9 \pm 1	959 \pm 96
3	50; CN _x -TiO ₂	7	EDTA	52960 \pm 5296	2.65 \pm 0.27	132 \pm 13	13217 \pm 1322
4	50; CN _x -TiO ₂	6	CA	7032 \pm 1380	0.35 \pm 0.07	21 \pm 3	2097 \pm 397
Variation of pH values (UV-vis irradiation)							
5	50; CN _x -TiO ₂	5	EDTA	111491 \pm 14138	5.57 \pm 0.59	262 \pm 39	26273 \pm 3989
6	50; CN _x -TiO ₂	6	EDTA	116953 \pm 11695	5.85 \pm 0.59	279 \pm 28	27928 \pm 2793
7	50; CN _x -TiO ₂	7	EDTA	52960 \pm 5296	2.65 \pm 0.27	132 \pm 13	13217 \pm 1322
Visible Light Irradiation ($I > 420$ nm)							
8	50; CN _x -TiO ₂	6	EDTA	26277 \pm 2628	1.31 \pm 0.13	63 \pm 6	6353 \pm 635
9	50; TiO ₂	6	EDTA	12327 \pm 1699	0.62 \pm 0.08	21 \pm 2	2093 \pm 222
10	50; CN _x	6	EDTA	2317 \pm 304	0.11 \pm 0.01	8 \pm 1	845 \pm 98
Visible Light Irradiation ($I > 455$ nm)							
10	50; CN _x -TiO ₂	6	EDTA	5152 \pm 1119	0.26 \pm 0.06	11 \pm 1	1096 \pm 175
11	50; TiO ₂	6	EDTA	993 \pm 193	0.05 \pm 0.01	—	—
12	50; CN _x	6	EDTA	— [#]	—	—	—
Control experiments (UV-vis irradiation)							
13	0; CN _x -TiO ₂	6	EDTA	—	0.35 \pm 0.06	10 \pm 2	—
14	50; CN _x -TiO ₂	6	H ₂ O [†]	25587 \pm 2558	1.28 \pm 0.13	88 \pm 9	8814 \pm 881
15	50; no CN _x -TiO ₂	6	EDTA	—	—	—	—
16	50; TiO ₂	6	EDTA	156308 \pm 15630	7.82 \pm 0.76	364 \pm 54	36418 \pm 5126
17*	50; CN _x -ZrO ₂	6	EDTA	3450 \pm 345	0.17 \pm 0.02	4 \pm 1	1054 \pm 229
18	50; CN _x	6	EDTA	14852 \pm 1485	0.74 \pm 0.07	63 \pm 7	6288 \pm 649

[#]No H₂ detected by GC measurements (limit of detection < 0.01%).

[†]A small amount of sacrificial electron donor TEOA was present in this experiment (16.5 μ L of 0.1 M TEOA H₂ase buffer solution).

*These experiments were carried out with 15 mg of CN_x-ZrO₂

Table S2. Solar light driven ($\lambda > 300$ nm) H_2 production using $\text{CN}_x\text{-TiO}_2$ (5 mg) in an aqueous EDTA solution (0.1 M, pH 6) with *Dmb* [NiFeSe]- H_2 ase (50 μmol) under standard conditions after 1 h of irradiation in the presence of different neutral density filters.

Entry	TON (4 h) $\pm \sigma$ / mol H_2 $\text{H}_2\text{ase}^{-1}$	H_2 (4 h) $\pm \sigma$ / μmol	Activity / $\mu\text{mol H}_2$ (g $\text{CN}_x\text{-TiO}_2$) $^{-1}$ h $^{-1}$	TOF $\pm \sigma$ / h $^{-1}$
No additional neutral density filter was employed				
1	105305 \pm 10531	5.3 \pm 0.5	281 \pm 28	28115 \pm 2812
A neutral density filter (Absorbance 50%) was employed				
2	96462 \pm 9646	4.8 \pm 0.5	228 \pm 23	22883 \pm 2289
A neutral density filter (Absorbance 80%) was employed				
3	46091 \pm 6145	2.3 \pm 0.3	95 \pm 12	9462 \pm 1164

*Vials were placed 10 cm closer to the light source thus may have been irradiated with a higher intensity of light than that used in standard experiments, thus a control with no filter was also measured. These measurements were performed in triplicate.

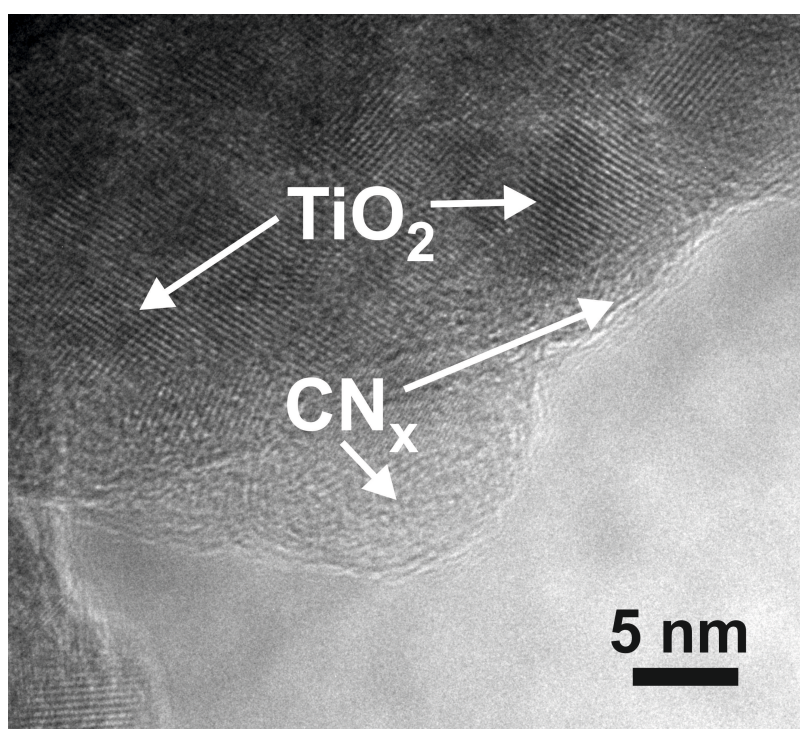


Figure S1. TEM of $\text{CN}_x\text{-TiO}_2$ material clearly showing CN_x on the TiO_2 surface. [M. Bledowski, L. Wang, S. Neubert, D. Mitoraj, R. Beranek, *J. Phys. Chem. C* **2014**, *118*, 18951-18961; M. Bledowski, L. Wang, S. Neubert, A. Ramakrishnan, R. Beranek, *J. Mater. Res.* **2013**, *28*, 411.]

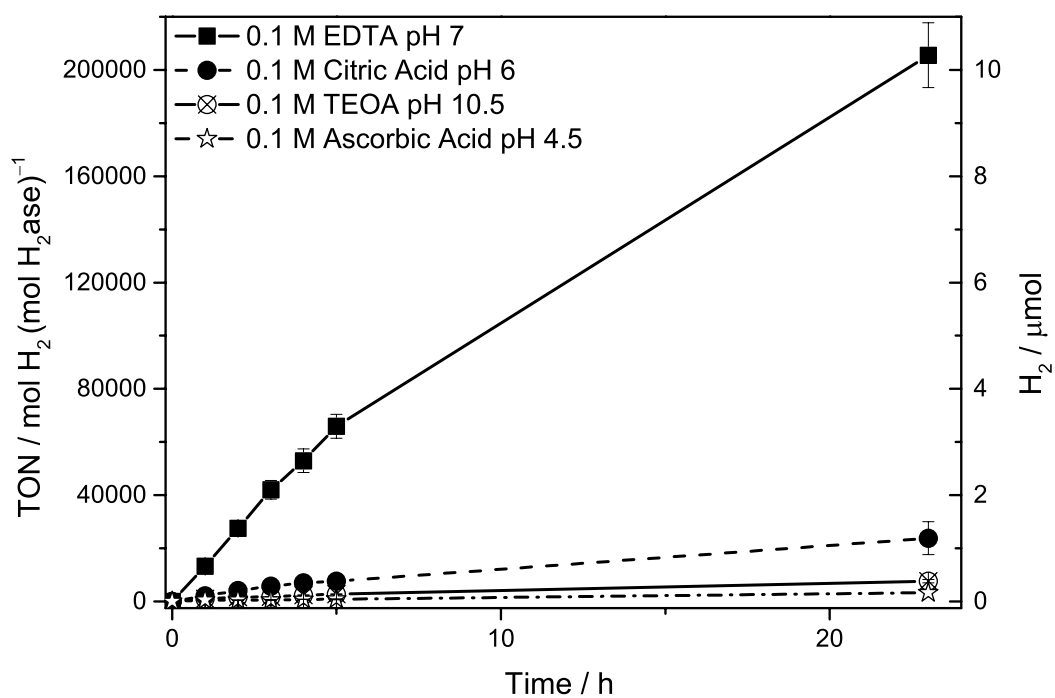


Figure S2. Photo-H₂ production with *Dmb* [NiFeSe]-H₂ase (50 pmol) with CN_x-TiO₂ (5 mg) in aqueous solutions with different sacrificial electron donors (0.1 M) during solar light irradiation (1 sun, 100 mW cm⁻², λ > 300 nm) at 25 °C.

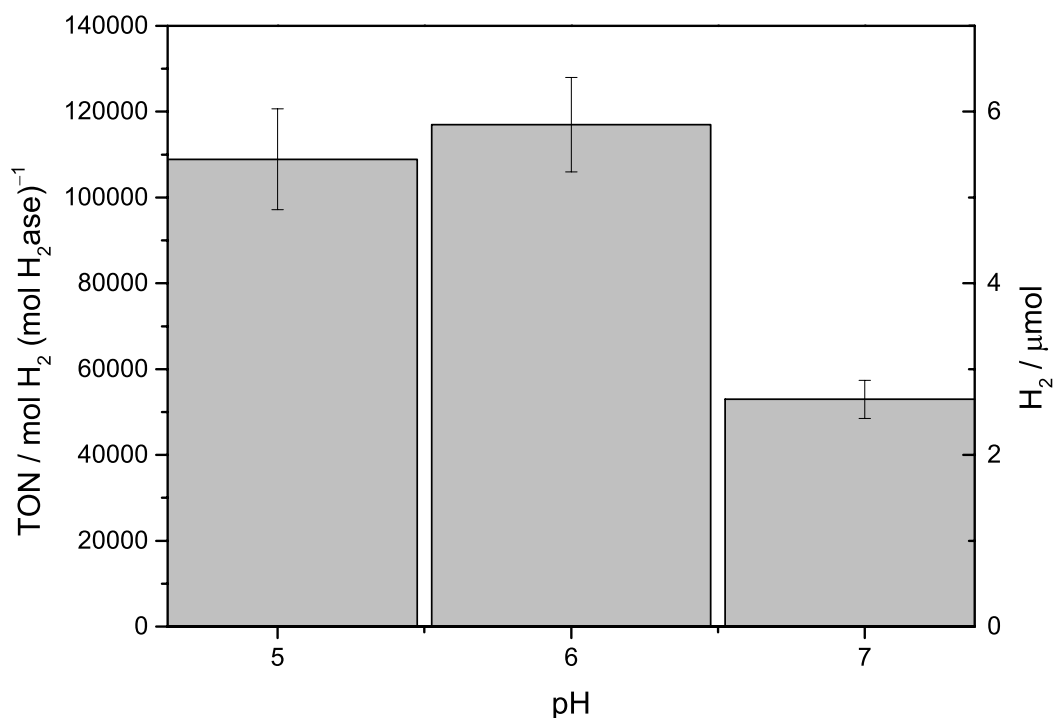


Figure S3. TON_{H₂ase} for photo-H₂ production with *Dmb* [NiFeSe]-H₂ase (50 pmol) in an aqueous EDTA solution (0.1 M) with CN_x-TiO₂ (5 mg) at different pH values obtained after 4 h of solar light irradiation (1 sun, 100 mW cm⁻², λ > 300 nm) at 25 °C.

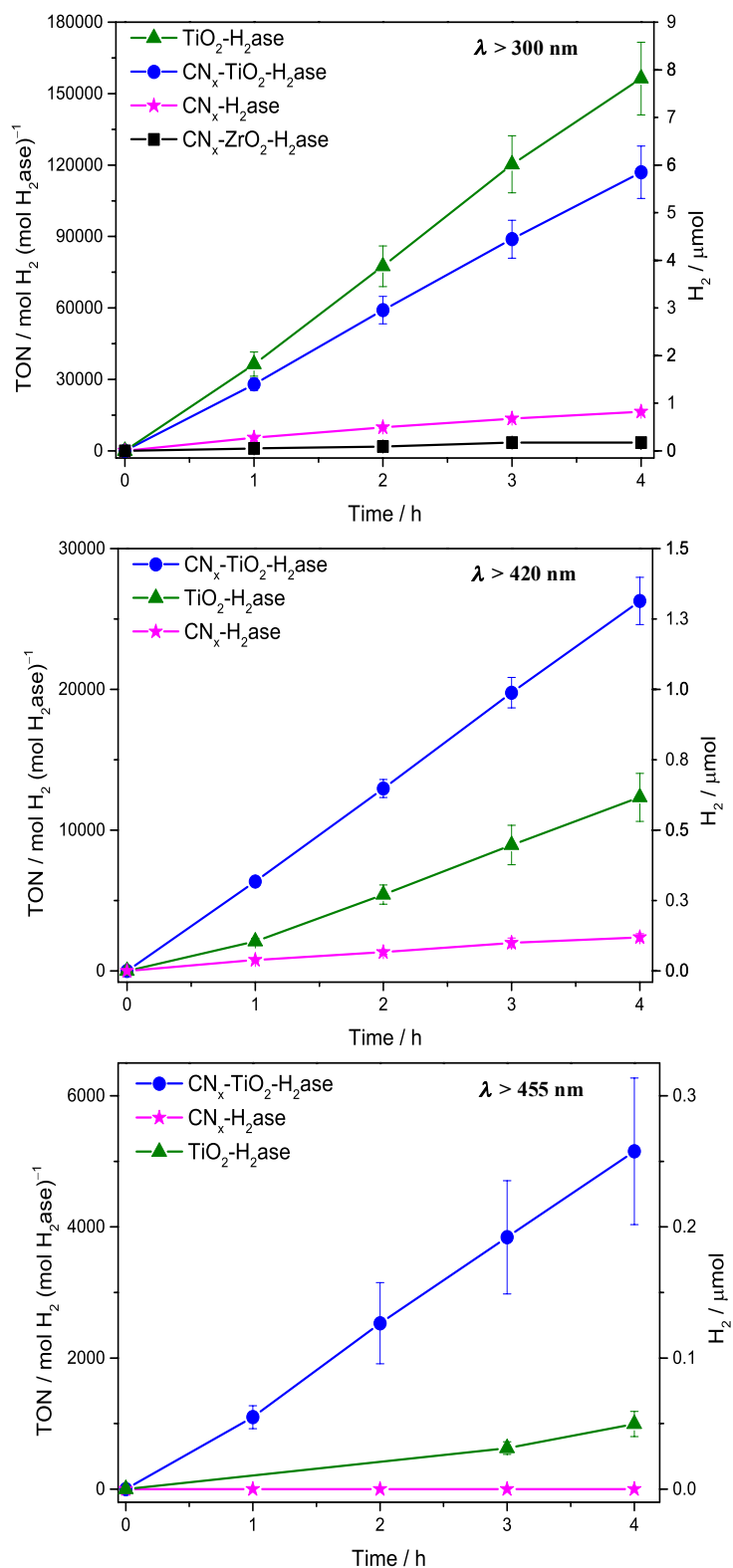


Figure S4. H_2 production under optimised conditions using *Dmb* [NiFeSe]- H_2ase (50 pmol) in EDTA (pH 6, 0.1 M, 3 mL) and $\text{CN}_x\text{-TiO}_2$, TiO_2 , CN_x , (5 mg) or $\text{CN}_x\text{-ZrO}_2$ (15 mg) under 1 sun irradiation with $\lambda > 300 \text{ nm}$, $\lambda > 420 \text{ nm}$ and $\lambda > 455 \text{ nm}$ light. Note that the amount of H_2 was below the detection limit after the first 2 h at $\lambda > 455 \text{ nm}$ with $\text{TiO}_2\text{-H}_2\text{ase}$.

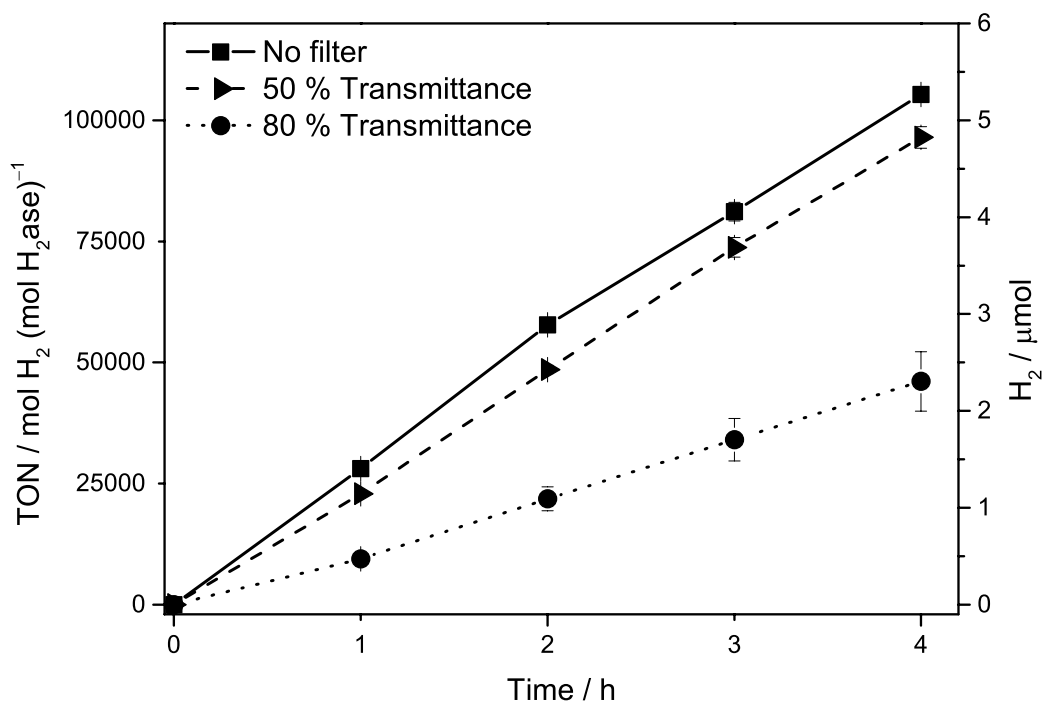


Figure S5. Photo-H₂ production using CN_x-TiO₂ (5 mg) in aqueous EDTA solution (0.1 M, pH 6) with *Dmb* [NiFeSe]-H₂ase (50 pmol) under 1 sun irradiation (100 mW cm⁻², λ > 300 nm, 25 °C) with the addition of different neutral density filters absorbing 50% and 80% of the incident light.

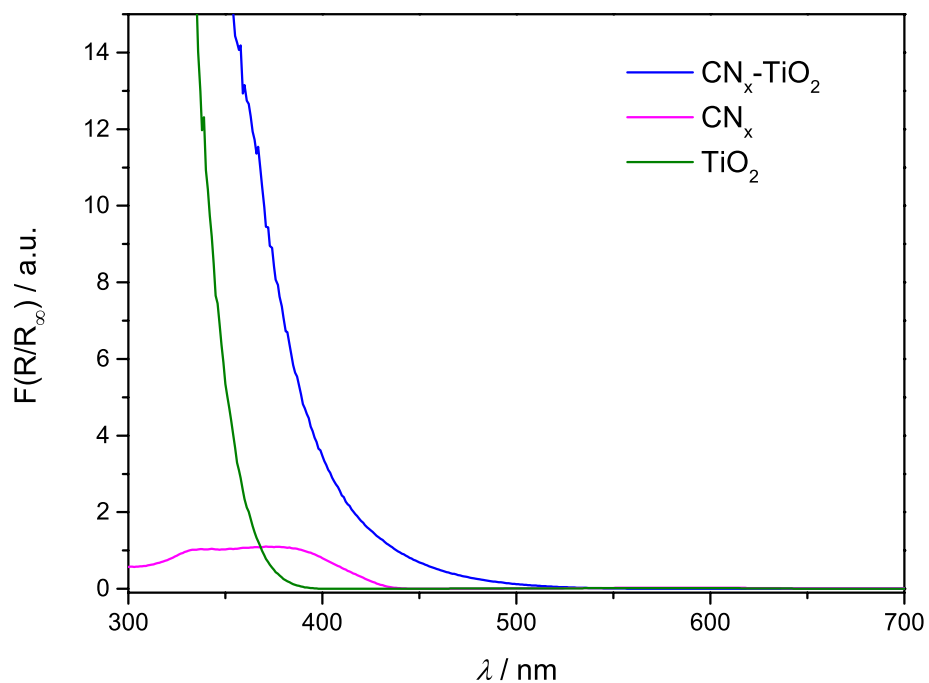


Figure S6. Diffuse reflectance UV-vis spectrum of CN_x-TiO₂, CN_x and TiO₂.

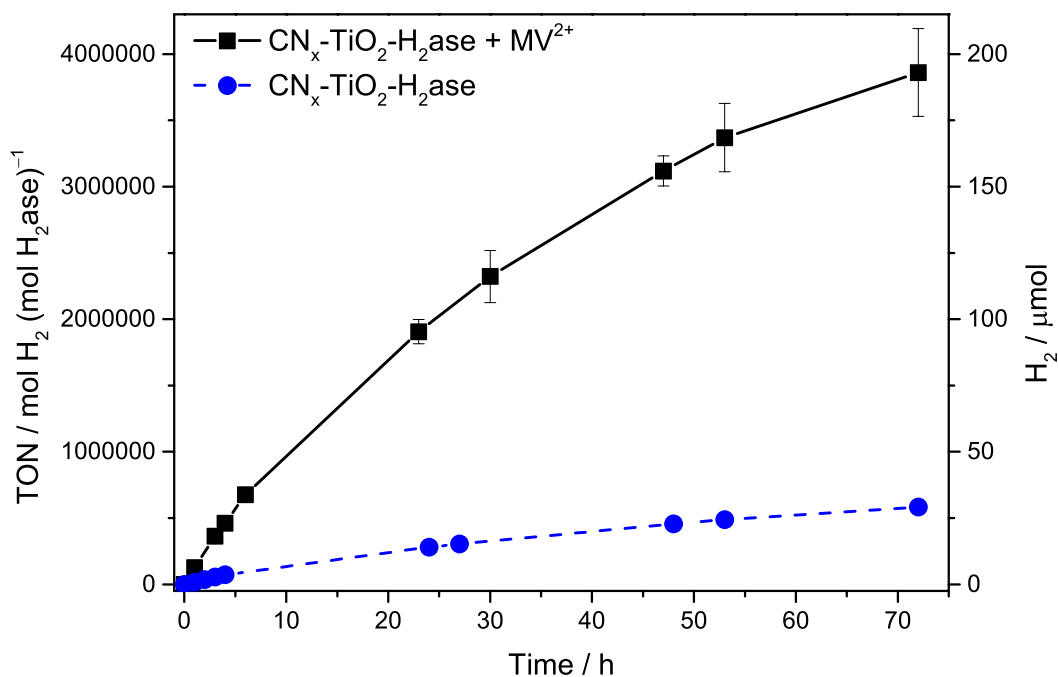


Figure S7. Long-term photo-H₂ production with *Dmb* [NiFeSe]-H₂ase (50 pmol) and CN_x-TiO₂ (5 mg) in aqueous EDTA solution (0.1 M, pH 6) in the presence and absence of methyl viologen (MV²⁺; 5 μmol) under solar light irradiation (1 sun, 100 mW cm⁻², λ > 300 nm) at 25 °C.

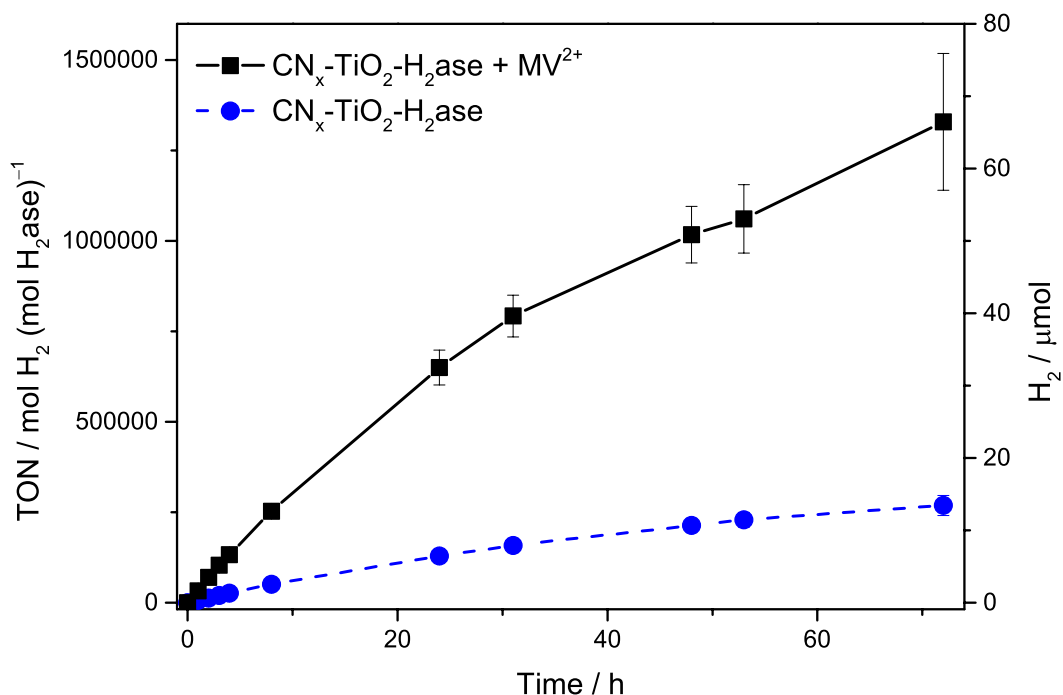


Figure S8. Long-term photo-H₂ production with *Dmb* [NiFeSe]-H₂ase (50 pmol) and CN_x-TiO₂ (5 mg) in aqueous EDTA solution (0.1 M, pH 6) in the presence and absence of methyl viologen (MV²⁺; 5 μmol) under visible light irradiation (1 sun, 100 mW cm⁻², λ > 420 nm) at 25 °C.

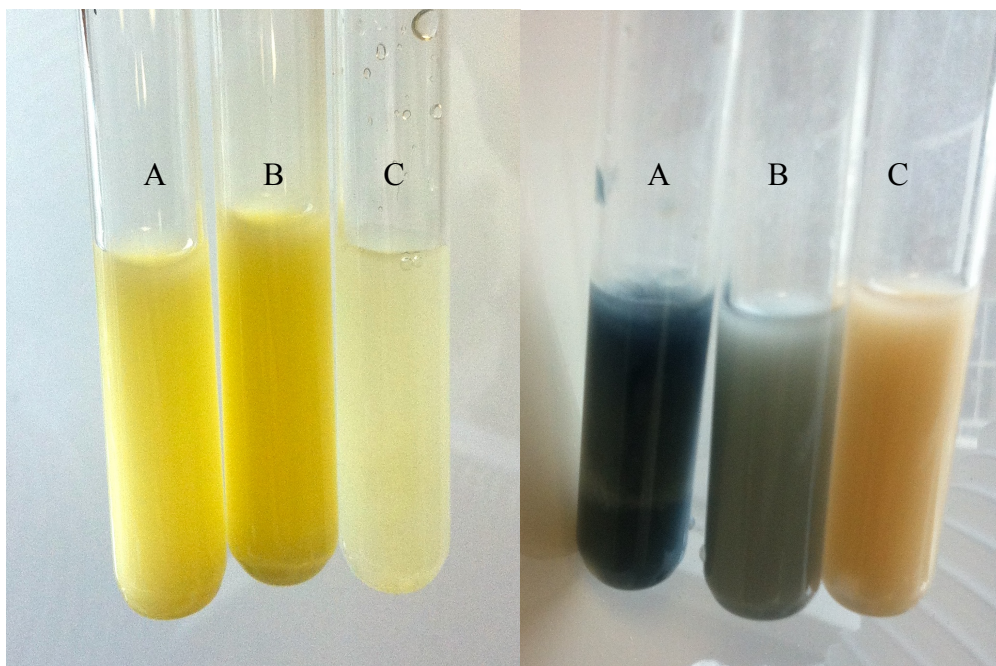


Figure S9. Colour of suspensions of (A) $\text{CN}_x\text{-TiO}_2\text{-H}_2\text{ase} + \text{MV}^{2+}$, (B) $\text{CN}_x\text{-TiO}_2\text{-H}_2\text{ase}$, (C) $\text{CN}_x\text{-H}_2\text{ase}$ before (left) and after 72 hours of solar light ($\lambda > 300 \text{ nm}$) exposure (right).

End of ESI